High blood pressure reduction reverses angiotensin II type 2 receptor-mediated vasoconstriction into vasodilation in spontaneously hypertensive rats.

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HIGH BLOOD PRESSURE REDUCTION REVERSES AT2 RECEPTOR-MEDIATED VASOCONSTRICTION INTO VASODILATION IN SPONTANEOUSLY HYPERTENSIVE RATS

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ABSTRACT

Background: We have previously shown that angiotensin II type 2 receptor (AT_2R) stimulation causes endothelium-dependent vasodilation which does not desensitize after chronic angiotensin II type 1 receptor (AT_1R) blockade, suggesting a role for AT_2R in antihypertensive treatment.

Material and Results: We recorded mean arterial pressure (MAP) and investigated AT_2R by western blot analysis, immunohistochemistry and function in isolated mesenteric resistance arteries (205 μm diameter) from Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) receiving for 4 weeks in drinking water: placebo, AT_1R blockade (candesartan, 2 mg/kg/d), angiotensin converting enzyme inhibitor (perindopril, 3 mg/kg/d), non-selective vasodilator (hydralazine, 16 or 24 mg/kg/d) or candesartan + hydralazine (16 mg/kg/d).

In precontracted isolated arteries AT_2R stimulation (Angiotesin II in the presence of candesartan) caused vasodilation in WKY rats (MAP=118 mmHg) and vasoconstriction in SHR (MAP=183 mmHg). In SHR treated with candesartan (MAP=146 mmHg) or hydralazine (16 mg/kg/d; MAP=145 mmHg) AT2R-induced contraction was reduced by 50%. In SHR treated with perindopril (MAP=125 mmHg), AT_2R stimulation induced a vasodilation. In SHR treated with hydralazine (24 mg/kg/d; MAP=105 mmHg) and in SHR treated with hydralazine (16mg/kg/d) + candesartan (MAP=102 mmHg) an AT_2R mediated-vasodilation was restored. Immunochemistry and Western-blot analysis showed that AT_2R expression, lower in SHR than in WKY was restored to normal level by treatments reducing arterial pressure in SHR.

Conclusions: Our results suggest that in resistance arteries of SHR 1) AT_2R is down regulated by hypertension and 2) specific and non-specific anti-hypertensive treatments restore AT_2R expression and vasodilator functions.

Keywords: angiotensin; receptors, vascular; vasodilation; hypertension.

INTRODUCTION

Angiotensin II (Ang II), the key effector of the renin-angiotensin-aldosterone system, has an important role in the control of blood pressure and blood volume. Angiotensin II activates at least two receptor types. ([1],[2]), the Ang II type 1 receptors (AT1R) the Ang II type 2 receptors AT2R([3],[4],[5]). Stimulation of AT2R undoubtedly induces relaxation in several vascular territories ([6],[7]). In most blood vessels AT2R-dependent relaxation is associated with activation of the bradykinin (BK) and/or nitric oxide (NO)/Guanosine 3', 5'-cyclic monophosphate (cGMP) pathway ([8],[9],[10],[11]). In vitro, the vasodilator role of AT2R is supported by evidence based on enhance Ang II-mediated vasoconstriction in the presence of AT2R blockade or in AT2R knockout mice ([12],[13],[14]). AT2R mRNA and protein expression was demonstrated in resistance arteries from normotensive rats ([15],16). We have previously shown in mesenteric resistance arteries from WKY, that AT2R is involved in NO-dependent flow-mediated dilation ([16]), whereas in SHR flow (shear stress) stimulation of the endothelium is associated to an AT1R and endothelin-1 type A receptor activation, thus counteracting endothelium-dependent dilation ([17],). Furthermore, acute administration of an AT2R inhibitor reversed both the acute antihypertensive effects and elevated level of BK, NO and cGMP in renal interstitial fluid caused by AT_1R blockade in renal wrap and salt-restricted rats ([18],[19]). Finally, we have recently shown the reproducibility of the vasodilator effect of AT_2R stimulation under acute and chronic AT_1R blockade ([20]), further supporting the assumption that AT_2R stimulation might play a role in the antihypertensive effect of AT_1R blocking drugs. Thus, we speculate that AT2R-stimulation, and hence vasodilation, might play a role in antihypertensive treatments, especially when AT1R blocking agents are used. Indeed, AT1R antagonists induce an important rise in circulating angiotensin II. AT2R might then be chronically overstimulated, participating to the maintenance of a vasodilation, particularly since this effect was not easily desensitized (20).

Thus, in the present study, we evaluated the vasomotor role of AT_2R in resistance arteries isolated from SHR (hypertensive conditions) and after various chronic antihypertensive treatments, including angiotensin-converting enzyme inhibition, AT1R blockade or a non-selective treatment, as well as AT2R expression and immunolocation. We hypothesized that AT2R-mediated dilation, which was impaired in untreated SHR, would be re-established in mesenteric arteries taken from treated SHR in parallel with reductions in blood pressure.

METHODS

Animal model

7-8 weeks old male WKY and SHR rats were separated into 8 groups receiving for 4 weeks in drinking water: WKY: placebo or AT1R antagonist (candesartan cilexetil, 2mg/kg/d), SHR: placebo, ACEI (perindopril, 3mg/kg/d), candesartan (2mg/kg/d), the non-selective antihypertensive drug (hydralazine, 2 groups: 16 or 24 mg/kg/d) or candesartan + hydralazine (16 mg/kg/d).

The protocol used was in accordance with the European Community standards on the care and use of laboratory animals (authorization no.00577).

MAP Measurement

After 4 weeks of treatment, rats were anesthetized with sodium pentobarbital (50mg per kg, i.p.). Mean arterial pressure (MAP) was measured in the right carotid artery using a catheter connected to a GOULD transducer and an analog-digital signal recording system (Biopac)(16,17)

Isolated mesenteric artery

A 3-4 mm-long segment of mesenteric artery (205 ± 11 µm, internal diameter measured at 75 mmHg in the absence of tone) was dissected, cannulated at both ends and mounted in a video monitored perfusion system ([21]) as we have previously described (16,17,20). Briefly, arteries were bathed in a physiological salt solution (PSS) maintained at 37°C, pH 7.4. The pO_2 was 160 mmHg and the pCO_2 37 mmHg (16,17,20). The artery was superfused (4 ml/min) and flow through the vessel was maintained at a rate of 100 µl/min, with intraluminal pressure set at 75 mmHg (20). Arterial diameter was measured (Living Systems Instrumentations, Burlington, VT, USA) and recorded continuously (Biopac, Lajolla, CA, USA). Vessels were allowed to stabilize for at least 30 minutes before drugs were added to the PSS superfusion. The integrity of the endothelium was assessed by testing the relaxing effect of acetylcholine (Ach, 1 µmol/L) after precontraction with phenylephrine (PE, 1 µmol/L). The arteries were then exposed to candesartan (100 nmol/L) for at least 30 minutes before exposure to Ang II while they were precontracted with PE (1 µmol/L) and serotonin (0.1 µmol/L) in order to induce a stable reduction in diameter. When this response had reached a plateau, a concentration-response curve to Ang II (0.1 nmol/L to100 nmol/L) or Ang II (100 nmol/L)

was performed and changes in diameter were measured. Ach (1 µmol/L) was used to completely dilate the vessels. A number of 8 rats was used in each group.

In separate series of experiments (n=5 per group), AT2R stimulation was repeated before and after application of one of the following drug: the cyclooxygenase inhibitor indomethacin (10 µmol/L), the thromboxane A2-PGH2 receptor blocker SQ29548 (10 µmol/L), the bradykinin B2 receptor blocker HOE140 (0.1 µmol/L), the endothelin receptor blocker bosantan (10 µmol/L).

Stimulation of AT2R was also performed before and after endothelium removal (5 second of air perfusion, n= 4 rats per group) in WKY rats, untreated SHR and in SHR treated with candesartan plus hydralazine (16 mg/kg/d).

Western Blot Analysis of AT2 Receptors

Western blot analysis of AT2R was performed in mesenteric resistance arteries of WKY rats and SHR (n=8 per group). Mesenteric arteries were also isolated from SHR treated with hydralazine (24mg/kg/d, for 18, 23, or 48 days, n=5 per group) or with candesantan + hydralazine (16mg/kg/d, n=5 per group). Mesenteric arteries were homogenized using a lysis buffer (1% sodium dodecyl sulfate SDS, 10 mmol/L Tris-HCl [pH 7.4], 1 mmol/L sodium orthovanadate , 2.5 mg/L leupeptin and 5 mg/L aprotin). Extracts were incubated at 25 °C for 30 minutes and then centrifuged (1 000g, 15 minutes, 14°C). Proteins concentration was determined using the Micro BCA Protein Assay Kit (Pierce). After denaturation at 100°C for 5 minutes, equal amounts of proteins (15 µg) were loaded on a 9% polyacrylamide gel and transferred to nitrocellulose membranes for 12 hours (40 V, 4°C). Membranes were blocked with 10% albumin bovine (BSA) in TBST (20 mmol/L Tris [pH 8.0], 150 mmol/L NaCl, and 0.1% Tween-20) for 1 hour and were then incubated with AT2R rabbit polyclonal antibody (dilution 1:100, Santa Cruz, Califonia, USA) in washing solution at room temperature for 20 hours. The membranes were then washed and incubated with the anti-rabbit horseradish peroxidase antibody (dilution 1 : 5000, Amersham Pharmacia Biotech, Orsay, France) for 1 h at room temperature. After 3 washes with TBS-T, immunocomplexes were detected by chemiluminescent reaction (ECL-kit; Amersham Pharmacia Biotech) using a computer based imaging system (Fuji LAS 1000 plus; Fuji Medical System). Quantification was performed by densitometric analysis.

Immunofluorescence Analysis of AT2 Receptors

Segments of mesenteric resistance arteries (n= 6 rats per group) were mounted in embedding medium (Miles, Inc), frozen in isopentane pre-cooled in liquid nitrogen, and stored at -80°C on transverse cross sections 7 μm thick. Sections were incubated with candesartan (30 min, 10 nmol/L, 25°C), then with fluorescent Angiotensin II (FITC-bound angiotensin II, 30 min, 10 pmol/L, 25°C, Molecular Probes). Fluorescence staining was visualized using confocal microscopy (Biorad MRC-600). Control experiments were performed after incubation with non-fluorescent Angiotensin II. Image analysis was performed using Histolab (Microvision, France). Briefly, pixels quantification was performed after separating the media and the endothelial layer. Data is given as percentage of control (fluorescence in WKY taken as 100%).

Statistical analysis

Results are expressed as means ± s.e. mean. The significance of the different treatments was determined by ANOVA or two tailed Student's paired t-test. P values less than 0,05 were considered to be significant. Number of rats was used for the analysis.

Drugs

Candesartan cilexetil was kindly provided by Astra-Zeneca (Sweden). Other products were purchased from Sigma.

RESULTS

Mean Arterial Pressure and AT2R Mediated dilation in isolated arteries

Figure 1 shows typical recordings obtained with mesenteric arteries isolated from a WKY rat (fig. 1A) and from SHR (fig. 1B). Mean arterial blood pressure was 118±8 mmHg (n=8) in WKY rats and 183±11 mmHg (n=8) in SHR. Isolated arteries were first incubated with

candesartan (100 nmol/L, 30 min) and pre-contracted with phenylephrine (54±4 µm, diameter decrease). Then, addition of Ang II (100 nmol/L) induced a significant dilation (24±3 µm, diameter increase) in control WKY. By contrast, in SHR the stimulation of AT2R led to a significant contraction (8±3 µm, diameter decrease, Fig. 1B). In both WKY and SHR the arteries were able to fully dilate when acetylcholine was added after ang II. Diameter changes in response to AT2R stimulation (dilation in WKY rats or contraction in SHR) were suppressed by the AT2R antagonist PD123319 (1 µmol/L). In the presence of candesartan and PD123319 Ang II produced no significant change in diameter (2±3 µm, n=5 in WKY and –1±2µm, n=4 in SHR).

The contraction induced by ang II (100 nmol/L) in arteries isolated from SHR was significantly reduced by indomethacin (10 µmol/L, 6±2 versus 12±3µm reduction in diameter, n=5), SQ29548 (10 µmol/L, 6±3 versus 14±3µm reduction in diameter, n=5) and by bosantan (10 µmol/L, 7±3 versus 16±3µm reduction in diameter, n=5). The combination of indomethacin (10 µmol/L) and bosantan (10 µmol/L) suppressed ang II-induced contraction in SHR (2±3 versus 14±3µm reduction in diameter, n=5). The bradykinin B2 receptor blocker HOE140 (0.1 µmol/L) did not significantly affect angII-induced contraction in SHR (13±3 versus 15±4µm reduction in diameter). In WKY rats angII-induced dilation was significantly reduced by L-NAME (4±2 versus 24±4 µm) and by HOE140 (6±3 versus 26±5 µm).

The stimulation of AT2R produced a concentration dependent dilation in arteries isolated from WKY rats and a concentration dependent contraction in arteries isolated from SHR (fig. 1C, n=8 per group).

Concentration dependent stimulation of AT2R (angII 0.01 to 100 nmol/L) was repeated in arteries isolated from WKY rats and SHR submitted to various treatments. Maximal responses to AT2R stimulation and mean arterial blood pressure determined in the different groups are shown in figure 2.

There was no significant difference in MAP and AT2R mediated dilation between control WKY rats (118±8 mmHg; 24±3 µm, diameter increase, n=8) and WKY rats treated with candesartan (108±9 mmHg, 19±5 µm diameter increase, n=4).

In SHR, candesartan partly depressed MAP (146±8 mmHg, n=4 versus 183±11 mmHg, n=8; $P<0.01$ versus SHR and $P<0.01$ versus WKY rats) and tended to reduce AT2R mediated

contraction, although this did not reach significance (8±3 μm versus 3±2 μm diameter decrease).

In SHR, treatment with perindopril reduced MAP (n=4, 125±6 mmHg, P<0.01) and AT2R stimulation induced a significant vasodilation (6±2 μm, diameter increase) which was significantly lower than AT2-induced dilation in WKY rats.

In hydralazine (16 mg/kg/d, n=4)-treated SHR, MAP decreased to a level that was still higher than in WKY rats (145±11 mmHg, n=6; P<0.01 versus SHR and P<0.05 versus WKY rats) and stimulation of AT2R induced a vasoconstriction (3±1 μm diameter decrease, n=6).

In SHR treated with a higher dose of hydralazine (24 mg/kg/d, n=4) MAP was reduced to a normal value (105±10 mmHg, NS versus WKY rats) and AT2R induced a significant vasodilation (27±7 μm, diameter increase, NS versus WKY rats).

In SHR treated with candesartan plus hydralazine (16 mg/kg/d) MAP was reduced to normal level (n=4, 102±9 mmHg, NS versus WKY rats) and AT2R induced a significant vasodilation (22±5 μm diameter increase, NS versus WKY rats).

Endothelium removal did not affect AT2R-dependent contraction in untreated SHR (12±3μm with endothelium versus 14±3 μm contraction without endothelium, n=4). On the other hand, AT2R-dependent dilation was abolished by endothelium removal in both untreated WKY (24±4 versus 3±2 μm dilation, n=4) and SHR treated with candesartan plus hydralazine (16 mg/kg/d: 26±4 versus 4±2 μm increase in diameter, n=4).

Western Blot Analysis of AT2R

In resistance arteries isolated from untreated WKY (n=8) rats and untreated SHR (n=8), as well as from SHR treated with hydralazine alone (24 mg/kg/d, n=5) or hydralazine (16 mg/kg/d, n=5) combined with candesartan, AT2R expression was quantified. Western-blot analysis showed that AT2R was significantly less expressed in SHR than in WKY rats (46%) in isolated mesenteric resistance arteries (fig. 3). Hydralazine (24mg/kg/d) gradually raised AT2R expression in SHR treated for 18, 23 and 48 days. After 48 days, AT2R expression was significantly higher than in SHR, but it remains significantly lower than in WKY (70%, Fig. 3). However, after 44 days of treatment, candesartan plus hydralazine (16 mg/kg/d) restored AT2R expression in SHR to a level equivalent to that found in WKY (96,7% versus 100%, Fig. 3).

Immunohistology analysis of AT2R

In WKY, immunofluorescence analysis of mesenteric resistance arteries, using confocal microscopy, indicated that AT2R was present in the endothelium, in the smooth muscle and in the adventitia. In the endothelium AT2R immunofluorescence was lower in SHR than in WKY rats (5±3% of WKY, NS, n=6 per group). In the media a significant fluorescence could be detected although it was lower than in WKY rats (32±6%, P<0.01 n=6). In SHR treated with candesartan + hydralazine (16mg/kg/d) immunofluorescence of AT2R was restored to a level equivalent to that found in WKY rats (compared to WKY: 88±11% in the endothelium and 81±18% in the media) (Fig. 4).

DISCUSSION

The present study demonstrates that AT2R stimulation induced a vasoconstriction in untreated SHR resistance arteries associated with a decrease in AT2R expression. Specific or non-specific anti-hypertensive treatments restored AT2R expression and its vasodilator function when the decrease in pressure was sufficient.

Recent evidence suggests that AT2R stimulation causes vasodilation in small resistance arteries in normotensive rats (6,7,11,16,17,20, [22]). This vasodilation may play an important role in the regulation of arterial blood pressure by increasing resistance arteries diameter. Vasodilation induced by AT2R stimulation has been described in several vascular territories and is usually associated to NO production by endothelial cells and cGMP production by smooth muscle cells ([23]). In some, but not all arteries investigated bradykinin B2 receptor activation is involved in AT2R-dependent dilation (9-11,22). Importantly, AT2R-mediated dilation does not desensitize, by contrast with AT1R-dependent contraction, supporting the assumption that AT2R dilation might have a role in the treatment of hypertension (20,[24]). Furthermore, in agreement with previous studies (21[25]) AT2R were found in resistance arteries using western blot and RT-PCR analysis. Using immuno-fluorescence AT2R were localized in endothelial and smooth muscle cells, as in previous studies in rat mesenteric arteries (16) and skeletal muscle arterioles ([26]).

We found that AT2R stimulation in SHR induced a vasoconstriction, in agreement with previous studies showing that in young hypertensive rats AngII-induced contraction was decreased by AT2R blockade (25). Interestingly, this effect involved stimulation of ET-1 and

probably thromboxane A2. We found that AT2R expression was lower in SHR resistance arteries than in WKY rats. However, a decreased expression cannot readily explain a reversal of dilation into contraction. The mechanism of this reversal remains to be discovered, but may involve a switch in signaling from constrictor to dilator mechanisms due to increased endothelial AT2R expression. Indeed, our immunohistological analysis of AT2R in mesenteric arteries from SHR showed undetectable AT2R labeling in the endothelium that was re-established with antihypertensive treatment that normalized blood pressure. In addition, AT2R-dependent contraction in SHR is not affected by endothelium removal whereas AT2R-dependent dilation is abolished in the absence of endothelium. Thus the difference in the type of response might reflect a change in AT2R expression between the endothelium and the smooth muscle. Nevertheless, due to the small size of the resistance arteries AT2R expression and mRNA level were not significantly decreased by endothelium removal in either SHR or WKY rats (unpublished data). In addition, cultured endothelial cells rapidly loose AT2R phenotype, thus preventing a study of AT2R expression in cultured endothelial cells from WKY rats or SHR.

Stimulation of the NO-cGMP pathway by AT2R has been initially shown in aortic cells ([27]). In the dog coronary circulation NO production by ang II is activated through both AT1R and AT2R stimulation ([28]). Several other evidences suggest that AT1R and AT2R may not always oppose each other, at least concerning NO production (23). In the mesenteric circulation we found that AT2R-dependent flow-mediated dilation requires NO production and that exogenous stimulation of AT2R in the same arteries produces dilation (16).

In WKY rats, candesartan did not significantly change MAP and AT2R mediated dilation was preserved, as we have previously reported (20). By contrast, candesartan partly decreased MAP and AT2R mediated vasoconstriction in SHR. Similarly, a low dose hydralazine, also partly reducing MAP, inhibited AT2R mediated vasoconstriction. Obviously, these treatments were not able to normalize MAP and to restore AT2R vasodilator function. Indeed, it was only when MAP was sufficiently reduced in SHR that AT2R mediated vasodilation was observed. First, using an ACEI inhibitor, MAP was decreased to a level similar to that observed in WKY. In this case, stimulation of AT2R induced a vasodilation. A further decrease in MAP, using a higher dose of hydralazine or candesartan plus hydralazine, was also associated with a vasodilator effect of AT2R stimulation.

Interestingly, the different groups studied allowed a correlation to be drawn between MAP and the type and amplitude of the response to AT2R-stimulation (figure 2). Thus, from high to low MAP, AT2R-stimulation moved progressively from contraction to dilation. In addition,

this effect was associated with a different AT2R expression. In SHR, AT2R expression was low in the wall of mesenteric arteries, compared to WKY. After MAP reduction in SHR, AT2R expression was restored to the level of WKY. However, it is difficult to determine if AT2R-mediated dilation is a cause or a consequence of the reduction in blood pressure. In the combined candesartan/ hydralazine group of SHR, the time course for AT2R expression, assessed by Western blots, was in parallel with the restoration of maximal AT2R-mediated dilation and normotension, which may indicate a primary role for AT2R. On the other hand, equivalent reductions in MAP caused by hydralazine were associated with maximal AT2R-mediated dilation despite suppressed AT2R expression. These discrepancies in AT2R abundance between treatments may reflect distinct AT2R locations within the vasculature. Indeed, more precise immunohistological analysis demonstrated that AT2R expression was located in the endothelial and smooth muscle cells in WKY, whereas, in SHR AT2R was not detectable in the endothelium. On the other hand, in SHR treated with candesartan plus hydralazine, MAP was restored, AT2R stimulation induced a vasodilation and AT2R expression was equivalent to that found in WKY. In this group, immunohistology of AT2R showed the presence of the receptor in both endothelia and smooth muscle cells. Thus we can speculate that the presence or absence of AT2R on endothelial cells has a key role in determining the type of response, at least in part, as the inhibition of the vasodilation in WKY by L-NAME does not uncover a vasoconstriction due to receptors located on the muscle, as revealed by immunohistology**.** We can also assume that MAP per se is the effector determining the type of response induced by AT2R stimulation (figure 2C).

In conclusion, in resistance arteries of SHR 1) AT2R is down regulated by hypertension and 2) specific and non-specific anti-hypertensive treatments restore AT2R expression and vasodilator functions. Whether or not this AT2R plasticity directly contributed to the blood pressure reduction, this AT2R dilator mechanism is likely to contribute to the maintenance of a vasodilator state during chronic treatment.

[1] Whitebread S, Mele M, Kamber B de Gasparo M. Preliminary biochemical characterization of two angiotensin II receptor subtypes. *Biochem Biophys Res Commun.* 1989;163:284–291.

[2] Chiu AT, Herblin WF, McCall DE, Ardecky RJ, Carini DJ, Duncia JV, Pease LJ, Wong PC, Wexler RR, Johnson AL, Timmermans P. Identification of angiotensin II receptor subtypes. *Biochem Biophys Res Commun.* 1989;165:196–203.

[3] Senbonmatsu T, Ichihara S, Price E, Gaffney FA, Inagami T. Evidence for angiotensin II type 2 receptor-mediated cardiac myocyte enlargement during in vivo pressure overload. *J Clin Invest.* 2000;106:R25-R29.

[4] Ichihara S, Senbonmatsu T, Price E, Ichiki T, Gaffney FA, Inagami T. Angiotensin II type 2 receptor is essential for left ventricular hypertrophy and cardiac fibrosis in chronic angiotensin II-induced hypertension. *Circulation.* 2001;104:346-351.

[5] de Gasparo M, Siragy HM. The AT2 receptor: fact, fancy and fantasy. Regul Pept. 1999; 31;81:11-24.

[6] Carey RM, Jin X-H, Siragy HM. Role of the angiotensin AT_2 receptor in blood pressure regulation and therapeutic implications. *Am J Hypertens*. 2001; 14: 98S-102S.

[7] Dimitropoulou C, White RE, Fuchs L, Zhang H, Catravas JD, Carrier GO. Angiotensin II relaxes microvessels via the AT_2 receptor and Ca2+-activated (Bkca) channels. *Hypertension*. 2001;37:301-307.

[8] Siragy HM, Carey RM. The subtype 2 (AT_2) receptor mediates renal production of nitric oxide in conscious rats. *J Clin Invest.* 1997;100: 264-269.

[9] Gohlke P, Pees C, Unger T. AT_2 receptor stimulation increases aortic cyclic GMP in SHRSP by a kinin-dependent mechanism. *Hypertension.* 1998;31: 349-355.

[10] Sosa-Canache B, Cierco M, Gutierrez CI, Israel A. Role of bradykinins and nitric oxide in the AT2 receptor-mediated hypotension. *J Hum Hypertens*. 2000;14 Suppl 1:S40-6.

[11] Bergaya S, Meneton P, Bloch-Faure M, Mathieu E, Alhenc-Gelas F, Levy BI, Boulanger CM. Decreased flow-dependent dilation in carotid arteries of tissue kallikrein-knockout mice. Circ Res. 2001;88:593-9.

[12] Hannan RE, Davis EA, Widdop RE. Functional role of angiotensin II AT2 receptor in modulation of AT1 receptor-mediated contraction in rat uterine artery: involvement of bradykinin and nitric oxide. *Br J Pharmacol*. 2003;140:987-95.

[13] Hein L, Barsh GS, Pratt RE, Dzau VJ, Kobilka BK. Behavioural and cardiovascular effects of disrupting the angiotensin II type 2 receptor gene in mice. *Nature.* 1995;377:744-747.

[14] Ichiki T, Labosky PA, Shiota C, Okuyama S, Imagawa Y, Fogo A, Niimura F, Ichikawa I, Hogan BLM, Inagami T. Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. *Nature.* 1995;377:748-750.

[15] Bonnet F, Cooper ME, Carey RM, Casley D, Cao Z. Vascular expression of angiotensin type 2 receptor in the adult rat: influence of angiotensin infusion. *J Hypertens*. 2001;19: 1075-1081.

[16] Matrougui K, Loufrani L, Heymes C, Levy BI, Henrion D. Activation of AT_2 receptors by endogenous angiotensin II is involved in flow-induced dilation in rat resistance arteries. *Hypertension*. 1999;34: 659-665

[17] Matrougui K, Lévy BI, Henrion D. Tissue angiotensin II and endothelin-1 antagonize the response to flow in mesenteric resistance arteries of spontaneously hypertensive rats. *Br J Pharmacol*., 2000;130 521-526.

[18] Siragy HM, De Gasparo M, Carey RM. Angiotensin type 2 receptor mediates valsartan-induced hypotension in conscious rats. *Hypertension*. 2000;35:1074-1077.

[19] Siragy HM, Carey RM. Protective role of the angiotensin AT_2 receptor in a renal wrap hypertension model. *Hypertension*. 1999; 33:1237-1242

[20] Widdop RE, Matrougui K, Levy BI, Henrion D. AT2 receptor-mediated relaxation is preserved after long-term AT1 receptor blockade. *Hypertension.* 2002;40:516-20.

[21] Halpern W, Kelley, M. In vitro methodology for resistance arteries. Blood vessels 1991;28: 245-251.

[22] Tsutsumi Y, Matsubara H, Masaki H, Kurihara H, Murasawa S, Takai S, Miyazaki M, Nozawa Y, Ozono R, Nakagawa K, Miwa T, Kawada N, Mori Y, Shibasaki Y, Tanaka Y, Fujiyama S, Koyama Y, Fujiyama A, Takahashi H, Iwasaka T. Angiotensin II type 2 overexpression activates the vascular kinin system and causes vasodilation. *J Clin Invest*. 1999;104: 925-935.

[23] Hannan RE, Gaspari TA, Davis EA, Widdop RE. Differential regulation by AT1 and AT2 receptors of angiotensin II-stimulated cyclic GMP production in rat uterine artery and aorta. *Br J Pharmacol.* 2004;141:1024-31.

[24] Widdop RE, Jones ES, Hannan RE, Gaspari TA. Angiotensin AT2 receptors: cardiovascular hope or hype? *Br J Pharmacol*. 2003;140:809-24.

[25] Touyz RM, Endemann D, He G, Li JS, Schiffrin EL. Role of AT2 receptors in angiotensin II-stimulated contraction of small mesenteric arteries in young SHR. Hypertension. 1999;33:366-72.

[26] Nora EH, Munzenmaier DH, Hansen-Smith FM, Lombard JH, Greene AS. Localization of the ANG II type 2 receptor in the microcirculation of skeletal muscle. *Am J Physiol*. 1998;275:H1395-403.

[27] Wiemer G, Scholkens BA, Wagner A, Heitsch H, Linz W. The possible role of angiotensin II subtype AT2 receptors in endothelial cells and isolated ischemic rat hearts. *J Hypertens.* 1993;11 Suppl 5:S234-5.

[28] Seyedi N, Xu X, Nasjletti A, Hintze TH. Coronary kinin generation mediates nitric oxide release after angiotensin receptor stimulation. *Hypertension*. 1995;26:164-70.

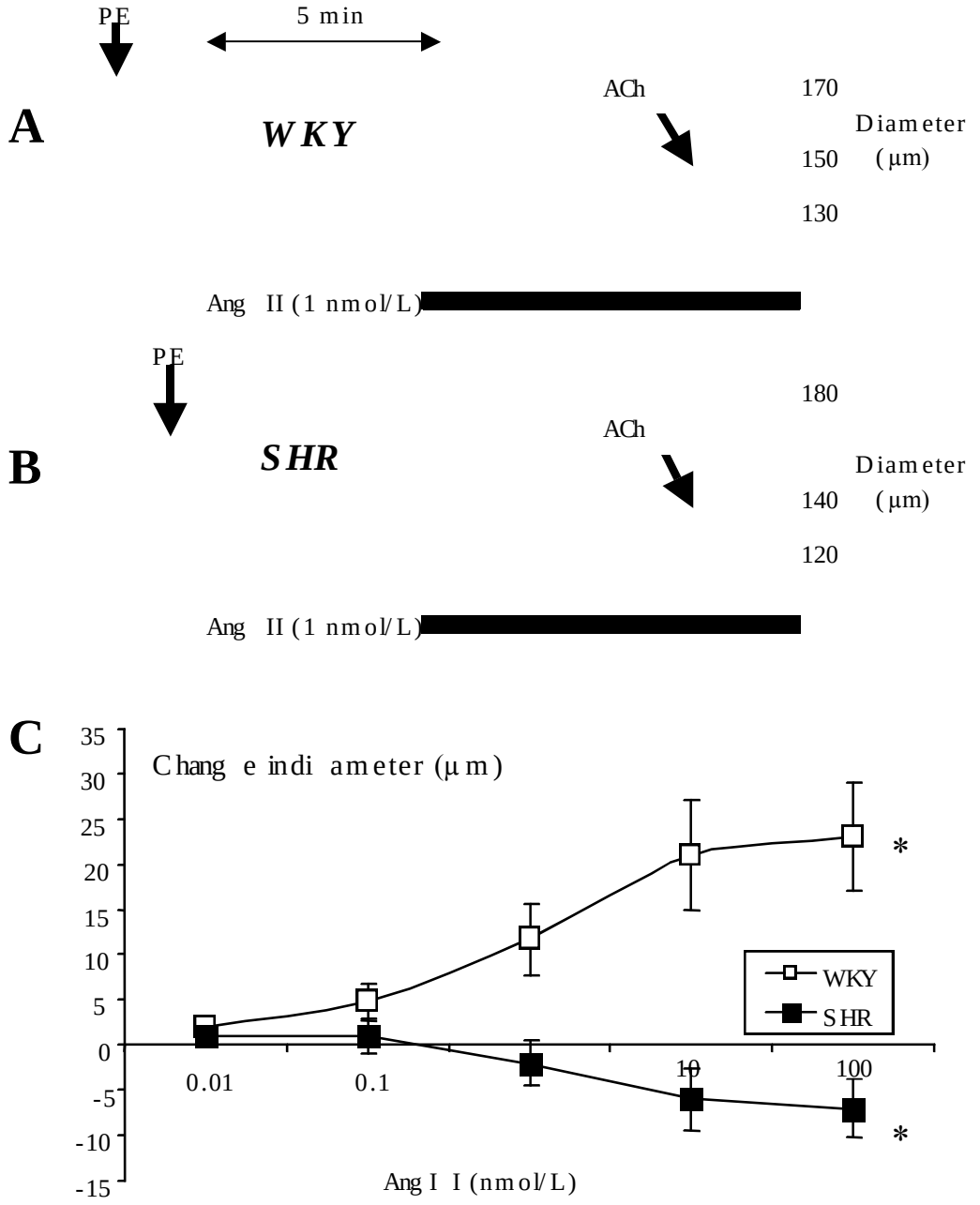
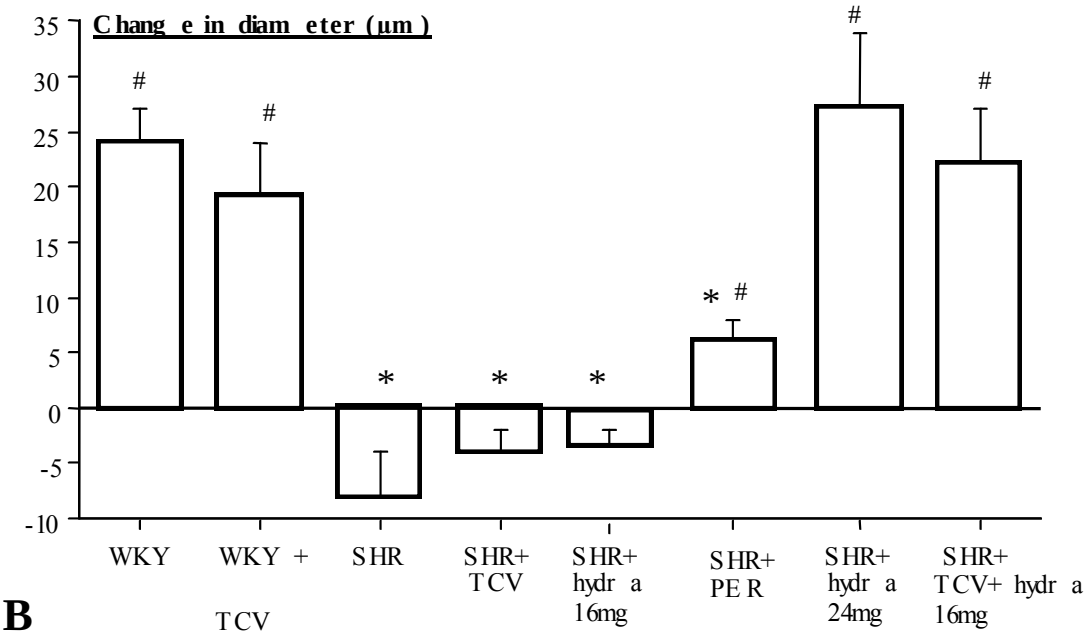


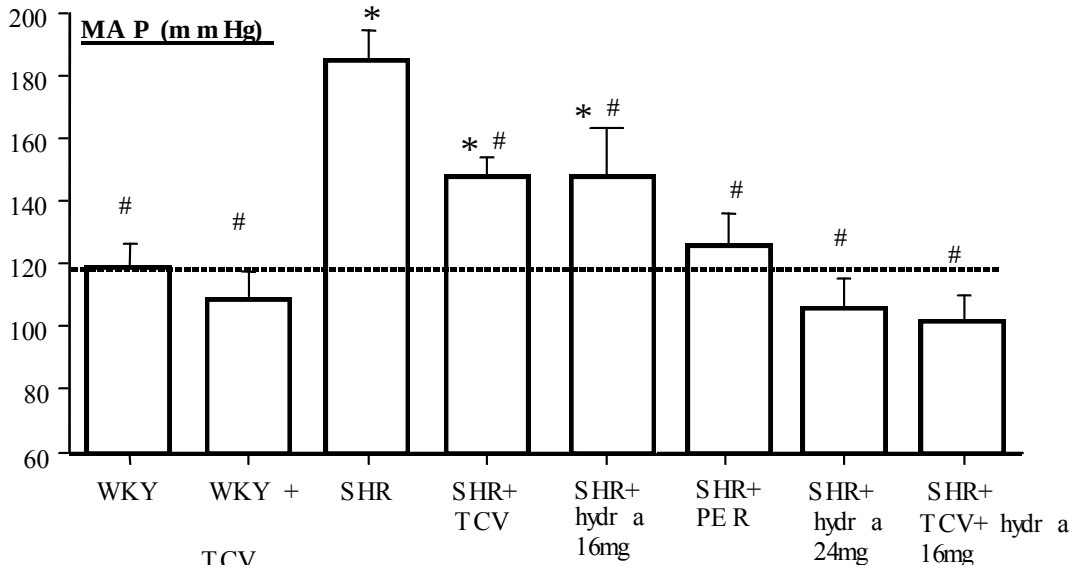
Figure 1: Typical recordings obtained in mesenteric arteries isolated from WKY (A) and SHR (B) rats and perfused under a pressure of 75 mmHg, a flow of 100 µl/min and in the presence of candesartan (100 nmol/L). After a precontraction with phenylephrine (PE), angiotensin II (Ang II, 100 nmol/L) was added to the bath containing the artery. Finally acetylcholine (1 µM) was added in order to fully relax the artery (n=8 per group).

The lower panel (C) shows concentration-response curves to ang II obtained in the conditions described above.

A


Change in diameter (µm)
35
30
25
20
15
10
5
0
-5
-10
WKY
WKY + TCV
SHR
SHR+ TCV
SHR+ hydra 16mg
SHR+ PER
SHR+ hydra 24mg
SHR+ TCV+ hydra 16mg

B


MAP (mmHg)
200
180
160
140
120
100
80
60
WKY
WKY + TCV
SHR
SHR+ TCV
SHR+ hydra 16mg
SHR+ PER
SHR+ hydra 24mg
SHR+ TCV+ hydra 16mg

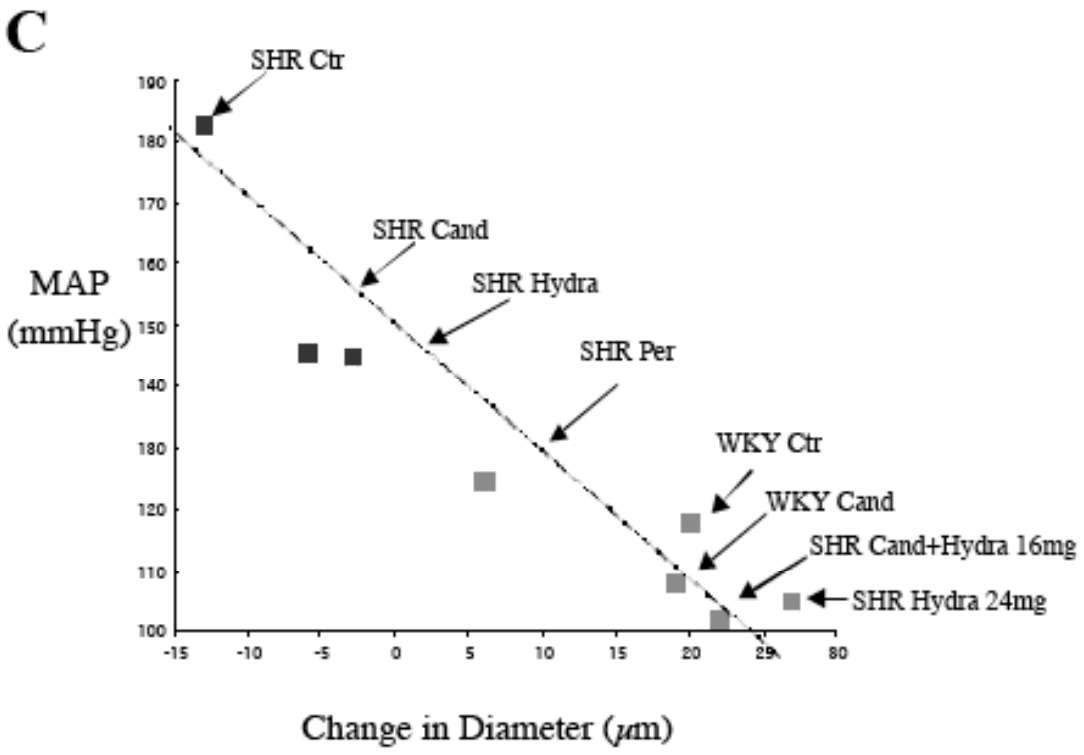


Figure 2:

Upper panel (A): Angiotensin II (Ang II, 100 nmol/L) mediated-dilation (diameter increase) or contraction (diameter decrease) in the presence of candesartan (100 nmol/L), obtained in isolated mesenteric arteries of the normotensive rats (WKY) or spontaneously hypertensive rats (SHR) perfused in an arteriograph under a pressure of 75 mmHg and a flow of 100 µl/min). Rats were treated for 4 weeks with candesartan (TCV, 2mg/kg/d), perindopril (PER, 3mg/kg/d), hydralazine (Hydra, 16 or 24 mg/kg/d), or candesartan + hydralazine (TCV 2mg/kg/d +Hydra 16mg/kg/d).

Middle panel (B): Meaning arterial pressure measured in the groups of rats described above. Mean ± SEM is presented (n=8 per group).

Lower panel (C): Relationship between mean arterial pressure (MAP) and AT_2R mediated-diameter-changes in resistance arteries (from data shown on panel A and B).

*P<0,05, versus control WKY;

#P<0,05, versus control SHR.

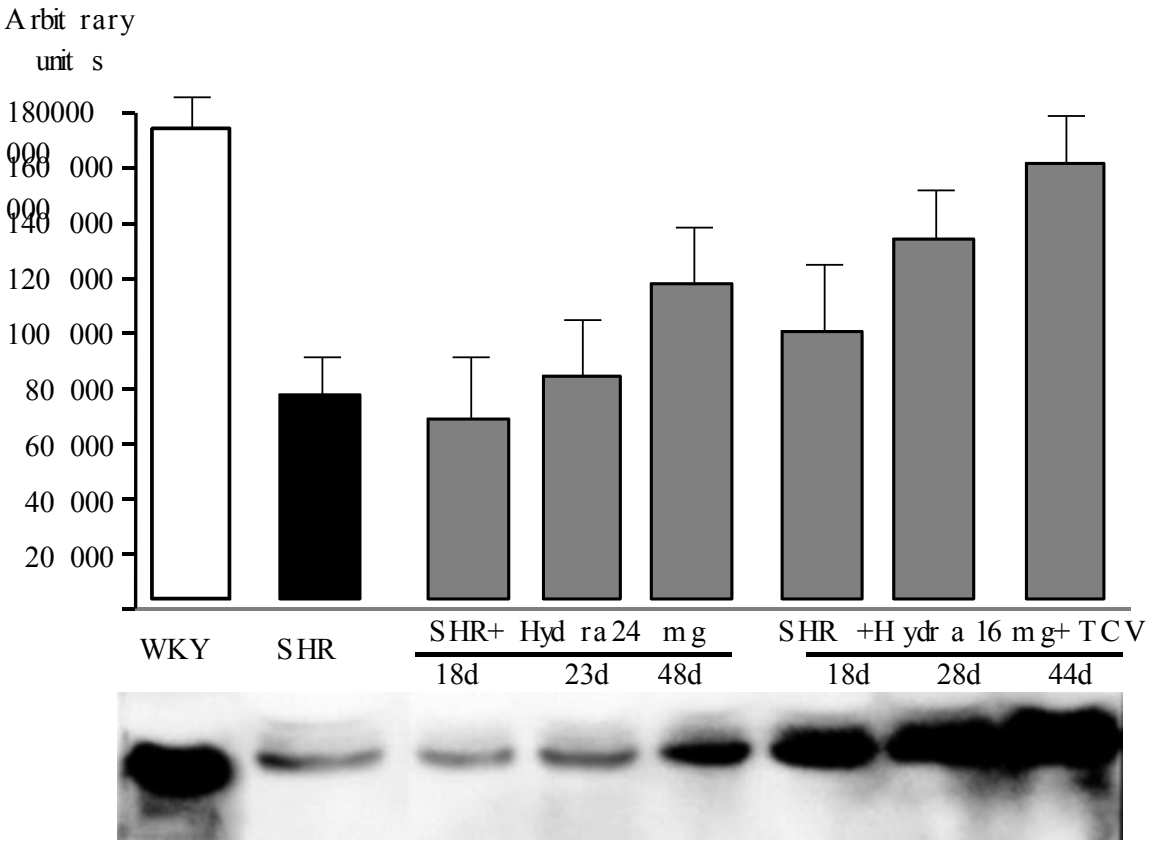


Figure 3: Western blot analysis of AT2R in mesenteric resistance arteries of normotensive (WKY, white bar, n=8) rats or spontaneously hypertensive rats (SHR, black bar, n=8). One group of SHR were treated with hydralazine (hydra, 24mg/kg/d) for 18, 23, or 48 days ,(n=5 per group). Another group of SHR was treated with candesantan + hydralazine (TCV + Hydra 16mg/kg/d, n=5 per group). Representative blots are shown below.

*P<0,05, versus control WKY;

#P<0,05, versus control SHR.

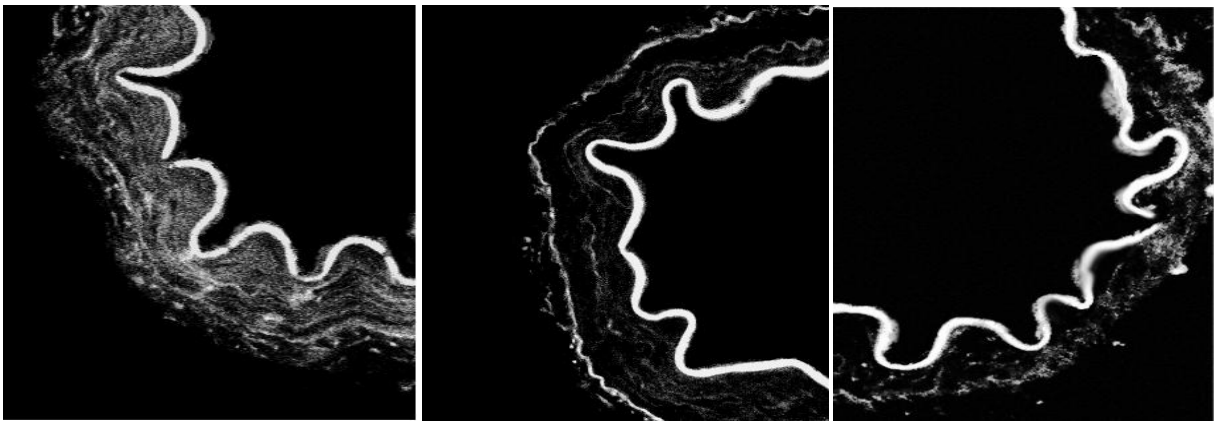


Figure 4: Confocal immuno-histological analysis of AT2R in mesenteric resistance arteries of the normotensive (WKY) rats, spontaneously hypertensive rats (SHR) or SHR treated with cadesantan (TCV, 2mg/kg/d) and hydralazine (Hydra, 16mg/kg/d). Each image is representative of 4 different arteries (6 rats).